

The effects of plant growth regulator (methyl jasmonate), salt (NaCl) stress and nutrient deficiency on biomass and hydrocarbon yields in *Helianthus annuus* cv. Munchkin (Asteraceae, Sunflowers)

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ABSTRACT

Sunflowers, *H. annuus* cv. Munchkin, were subjected to 5 different treatments (spraying twice with 100 mM methyl jasmonate (MeJA), stress by watering with 30 mM, 120 mM and 240 mM salt (NaCl) water, and nutrient stress, by growing in sand) to determine their effects on leaf biomass, free hydrocarbons (HC) concentrations, and yields of HC/ g biomass. Biomass was significantly larger than the control for plants sprayed twice with methyl jasmonate (100 µM, each) and harvested 7 days later. Increasing stress by watering with increasingly high concentrations of salt, resulted in slower growth and less biomass. Growing the Munchkin plants in sand (very low nutrients) produced stunted plants with very small amounts of biomass, but with a high % HC yields. Salt concentrations of 30 mM and 120 mM decreased the % HC yields, but 240 mM plants had, statistically, the same % HC yields as the control. Total hydrocarbon yield (as g HC/ g DW 10 leaves) was largest in MeJA spraying, followed by the control, with no significant differences among salt concentrations, followed by very small yields under nutrient stress (sand treatment). Published on-line www.phytologia.org *Phytologia* 101(3): 194-199 (Sept 21, 2019). ISSN 030319430.

KEY WORDS: *Helianthus annuus*, Sunflower, methyl jasmonate, effects on hydrocarbon yields.

Soil salinization is said to affect over 100 countries in the world with varying levels and agricultural responses. High soil salinity levels in farmland can cause plant stress responses so detrimental that these lands are unusable. These unusable lands have an impact on not only agricultural production in these areas, but environmental health and economic welfare as well (Renegasamy 2006). Salt (NaCl) is an abiotic compound that is hydrophilic and as such, attracts water and retains it in the soil resulting in less available water for plants to absorb (Renegasamy, 2006). Plants then go into a drought stress-response phase which may change the metabolites. Responses include a myriad of mechanisms causing stunted growth, decreased dry weight, and a reduced number of leaves (Khalid and Ahmed 2017).

Salt stress has been found to increase secondary metabolite production in various plants such as *Stevia rebaudiana* Bertoni, *Apocyni veneti* Folium, and *Nigella sativa* L (Chen et al. 2013; Khalid 2017; Chen et al. 2018).

Studies on the effects of salt solutions as the water source for growing *Chrysanthemum Xmorifolium* showed stunted plants in all NaCl test groups and the reduction of dry shoot weight in some groups (van Iesrel and Lee 2008).

Salt tolerance was tested on coastal and inland *Grindelia camporum* and *G. stricta* (Asteraceae) using concentrations ranging from 5mM to 550 mM, with the highest being near that of seawater (600 mM). Plants subjected to higher salt concentrations had significantly decreased biomass (Ravetta, D., et. al., 1997). Interestingly, crude resin content (%) declined in the 125 mM treatment, but increased in the 250 mM treatment after 84 days (Ravetta et. al. 1997). Plants recognize stress at a cellular level and

may respond by producing different amounts of secondary metabolites. such as hydrocarbons, phenyl amides, and polyamines (Ramakrishna and Ravishankar 2011).

In a paper on the induction of sesquiterpene lactone (STL) defenses in *Helianthus annuus*, by methyl jasmonate (MeJA), Rowe, Ro and Rieseberg (2012) reported MeJA treated sunflower plants had a lower STL production and lower glandular trichome density. This is in contrast to other studies that have found MeJA to induce increased concentrations of terpenoids in cotton (*Gossypium hirsutum*, Opitz, Kunert and Gershenzon, 2008), *Tanacetum parthenium* (Majdi et al. 2015) and see review on the roles of MeJA in plants by Browse (2005).

Defense chemicals are both constitutive and inducible defenses (see Wittstock and Gershenzon, 2002 for discussion). Recently, we reported (Adams et al. 2017c) that progeny of high hydrocarbon (HC) yielding sunflower (*H. annuus*) populations displayed much reduced HC yields when grown in greenhouse conditions. We reported the percent HC (greenhouse / field grown HC yields) decreased to 45.9, 55.6 and 78.3%. In addition, g HC / g DW weights of leaves were very reduced to from 17.9 g tp 6.1 g when plants were grown in a greenhouse. It appears that biotic and abiotic factors in natural populations can have large effects on HC yields.

The purposes of the present paper are to report the effects of plant growth regulator (methyl jasmonate, MeJA), salt (NaCl) stress and nutrient deficiency on biomass, % HC yields and HC yields as gHC/ g DW leaves.

This report is a part of a continuing study on the development of sunflowers as a source for natural rubber and bio-fuels from the biomass (Adams et al., 1986; Adams and Seiler, 1984; Adams and TeBeest, 2016; Adams et al. 2016; Adams and TeBeest, 2017; Adams et al. 2017a,b,c; Adams et al. 2018a,b,c; Pearson et al., 2010a,b; Seiler, Carr and Bagby, 1991,).

MATERIALS AND METHODS

Seeds of *H. annuus* cv. Munchkin were obtained from Sunflower Selections, Inc., Woodland, CA. Seeds were planted in 6 " square plastic pots using Miracle Grow® potting soil. Plants were grown in a growth chamber with LED lighting approximately equal to daylight for 16 hr light, 8 hr dark cycles and watered as needed.

Ten plants were used for each treatment (Table 1). Munchkin seeds were planted on Jan. 7 in soil saturated with tap water. All plants were watered with 200 ml tap water on Jan. 17, and 21, then with 300 ml tap water on Jan. 25, 29, and Feb. 1. Control plants were watered with 300 ml tap water, and salt (NaCl) treatments were watered with 300 ml of 30 mM, 120 mM and 240 mM of salt solutions on Feb. 4, 7, 12, 14. On Feb. 18 all plants (control, salt treatment) were watered with 300 ml of tap water and 10 leaves were harvested from each plant on Feb. 21.

Concurrent with the salt concentration experiment, 10 plants were grown in a low-nutrient soil mixture consisting of sand/ potting soil (7:1). Munchkin seeds were planted on Jan. 7 in soil saturated with tap water. Due to the fine texture of the sand and slow growth, the plants were not watered on Jan. 17, or Jan. 21. Plants were watered with 200 ml of tap water on Jan. 25, 29, Feb. 1, 4, 7, 12, 14, and 18. Plant leaves (10) were harvested on Feb. 21 (4 days after last watering).

In addition, 10 plants were grown with the aforementioned control and watered exactly as the control. On Feb. 22 these 10 plants were sprayed with 100 μ M methyl jasmonate with a hand sprayer until each leaf was wet then let dry, then sprayed again. Leaves were harvested on March 1, 7 days after spraying.

The 10 lowest growing, non-yellowed, mature leaves were collected. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Leaves were ground in a coffee mill (1mm). 3 g of air-dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar was sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was filtered through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pre-weighed aluminum pan with concentrated hydrocarbon extract was weighed and tared. Extraction of identical samples by shaking and soxhlet (8 hr) yielded a correction factor of 1.9 (soxhlet yield/ shaking yield), which when corrected to oven dry weight basis (ODW) by 1.085 resulted in a total correction factor of 2.06.

ANOVA and SNK (Student Newman-Keuls) multiple range tests were programmed following the formulations in Steel and Torrie (1960).

RESULTS

Table 1 shows the results from the treatments ANOVA and SNK statistical analyses. Biomass was highly significantly higher for methyl jasmonate (MJAS) sprayed plants. This is in contrast to a previous study (Adams and Johnson 2019) that reported biomass in Munchkin was not affected by the application of a single spraying of 100 µM MeJA, and harvested after 2 days, and 4 days. However, after 14 days, biomass was significantly larger, as the plants recovered from MeJA treatment and began to grow. It appears that the application of 2 sprays of 100 µM methyl jasmonate seems to initiate growth.

Table 1. Comparison of dry weight (10 leaves), percent HC yields, and g HC/ g DW 10 leaves for cv. Munchkin, subjected to 5 treatments and analyzed after 4 days. Mean values with the same suffix letter are not significantly different (P= 0.05).

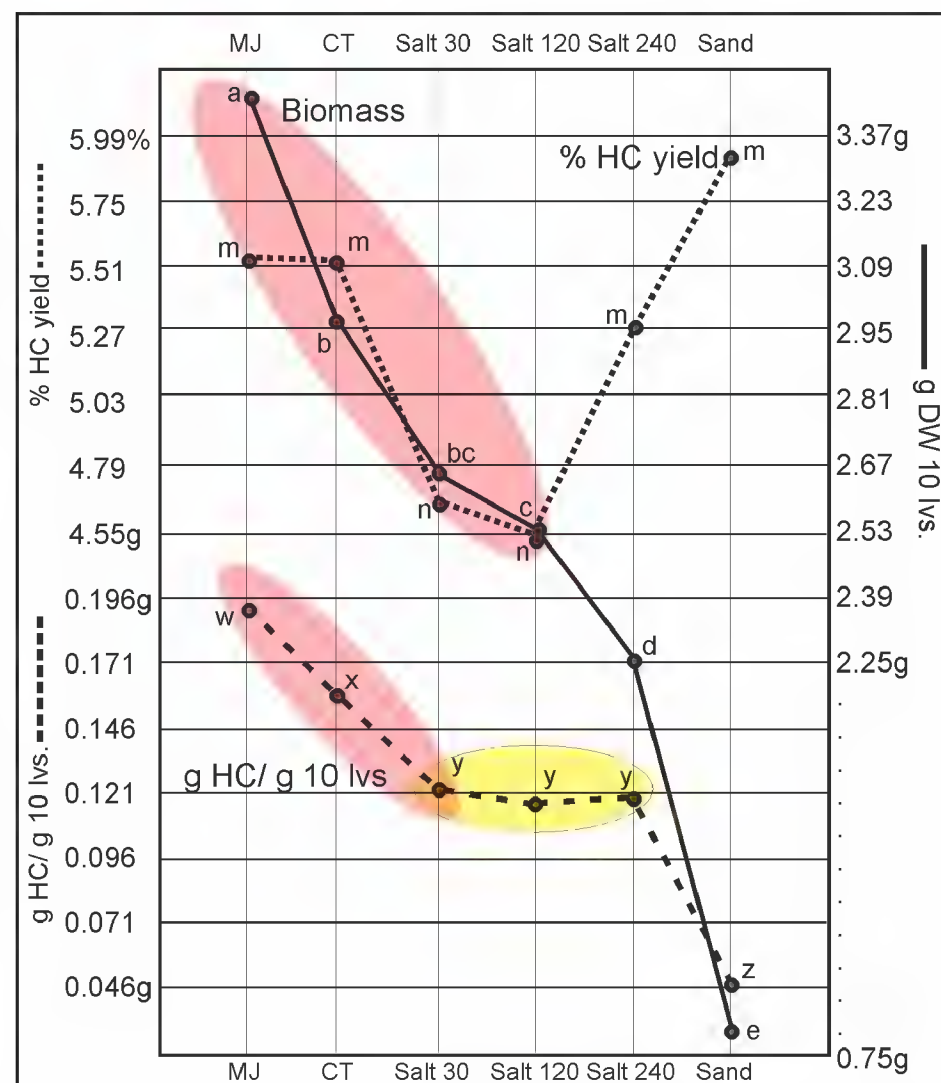
	MJ	CT	salt 30 mM	salt 120 mM	salt 240 mM	sand	F ratio significance
Biomass, g DW 10 leaves	3.47a	2.83b	2.66bc	2.54c	2.28d	0.77e	F= 126.9 P = 0.12 ⁻⁹ ***
	sand	MJ	CT	salt 240 mM	salt 30 mM	salt 120 mM	F ratio significance
% HC yield	5.96m	5.58m	5.53m	5.25m	4.63n	4.55n	F= 8.36 P = 0.38 ⁻⁴ ***
	MJ	CT	salt 30 mM	salt 240 mM	salt 120 mM	sand	F ratio significance
g HC/ g DW 10 leaves	0.193w	0.155x	0.123y	0.119y	0.116y	0.046z	F= 72.60 P = 0.67 ⁻⁹ ***

Increasing stress by watering with increasingly high concentrations of salt (NaCl) resulted in slower growth and less biomass (Table 1). Growing the Munchkin plants in sand (very low nutrients) produced stunted plants with very small amounts of biomass, but with a high % HC yields (Table 1). Salt concentrations of 30 mM, and 120 mM decreased the % HC yields, but 240 mM plants had, statistically, the same % HC yields as the control. This seems due to the low biomass production.

Hydrocarbon yield (as g HC/ g DW 10 leaves, Table 1) is the product of biomass x % HC yield and was largest in MeJA, followed by the control, with no significant differences among salt concentrations, followed by very low biomass in the nutrient stress (sand) treatment.

Graphing the yields by treatment reveals some interesting patterns (Fig. 1). Biomass, % HC yields, and g HC yield all decline from MeJA to control, 30 mM, and 120 mM salt (red ellipses, Fig. 1). However, at 240 mM salt (Fig. 1), the % HC yield increases. A similar trend is seen in the sand treatment (nutrient deficiency) with increased % HC yield and very low biomass (Fig. 1).

Figure 1. Graphs of dry weight (10 leaves), percent HC yields, and g HC/ g DW 10 leaves for Munchkin subjected to 5 treatments. Means with the same letter superscripts are **not** significantly different ($P= 0.05$). Similar trends are noted by the red ellipses. The yellow ellipse highlights the uniform HC yields in various salt concentrations. See text for discussion.



It is interesting that total g HC yields are not significantly different among the wide range of salt concentrations utilized in this study (yellow ellipse, Fig. 1). Considering that seawater is approximately 600 mM salinity, the 240 mM salt water is a very high salt concentration (~ 40% of seawater). These results suggest (ignoring environmental effects, etc.) that cultivation using available brackish water, that is common the western US, might be feasible. Although less biomass would be produced, total g HC yields (yellow ellipse, Fig. 1) are not significantly different. Of course, if biomass were used for cellulosic digestion to produce fuel and/ or chemical feedstocks, the use of high amounts of salty water might not be the best economic practice. In addition, irrigation with saline water on the arid lands in the southwest US would hasten the accumulation of salt, rendering the soil unusable.

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LITERATURE CITED

- Adams, R. P. and G. J. Seiler. 1984. Whole plant utilization of sunflowers. *Biomass* 4: 69-80.
- Adams, R. P., M. F. Balandrin, K. J. Brown, G. A. Stone and S. M. Gruel. 1986. Extraction of liquid fuels and chemical from terrestrial higher plants. Part I. Yields from a survey of 614 western United States plant taxa. *Biomass* 9: 255-292.
- Adams, R. P. and A. K. TeBeest. 2016. The effects of gibberellic acid (GA3), Ethrel, seed soaking and pre-treatment storage temperatures on seed germination of *Helianthus annuus* and *H. petiolaris*. *Phytologia* 98: 213-218.
- Adams, R. P., A. K. TeBeest, B. Vaverka and C. Bensch. 2016. Ontogenetic variation in hexane extractable hydrocarbons from *Helianthus annuus*. *Phytologia* 98: 290-297
- Adams, R. P. and A. K. TeBeest. 2017. The effects of different concentrations of gibberellic acid (GA3) on seed germination of *Helianthus annuus* and *H. petiolaris* *Phytologia* 99: 32-35.

- Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet, C. Parker and D. Thornburg. 2017a. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). *Phytologia* 99: 1-10.
- Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet and D. Thornburg. 2017b. Geographic variation in volatile leaf oils (terpenes) in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). *Phytologia* 99: 130-138.
- Adams, R. P., A. K. TeBeest, T. Meyeres and C. Bensch. 2017c. Genetic and environmental influences on the yields of hexane extractable hydrocarbons of *Helianthus annuus* (Asteraceae, Sunflowers). *Phytologia* 99: 186-190.
- Adams, R. P. and S. T. Johnson. 2018. The effects of methyl jasmonate on the growth and yields of hydrocarbons in *Helianthus annuus* (Asteraceae, Sunflowers). *Phytologia* 100: 177-182.
- Adams, R. P., A. K. TeBeest, S. McNulty, W. H. Holmes, J. A. Bartel, M. Corbet, C. Parker, D. Thornburg and K. Cornish. 2018a. Geographic variation in natural rubber yields in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). *Phytologia* 100: 19-27.
- Adams, R. P., Matt Lavin and Gerald P. Seiler. 2018b. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers) II. *Phytologia* 100: 153-160.
- Adams, R. P., Matt Lavin, Steve Hart, Max Licher and Walter Holmes. 2018c. Screening hydrocarbon yields of sunflowers: *Helianthus maximiliani* and *H. nuttallii* (Asteraceae). *Phytologia* 100: 161-166.
- Browse, J. 2005. Jasmonate: an oxylipin signal with many roles in plants. *Plant Hormones* 72: 431-456.
- Chen, A., Li, D., Wu, W., Yi, B. and Zeng, J. 2013. Effects of salt stress on the growth, physiological responses, and glycoside contents of *Stevia rebaudiana Bertoni*. *Journal of Agricultural Food Chemistry* 61: 5720.
- Chen, C., Chen, J., Chen, S., Liu, X., Liu, Z., Shi, J., Tan, M., Wang, C. and Zou, L. 2018. Variations in physiology and multiple bioactive constituents under salt stress provide insight into the quality evaluation of *Apocyni Veneti Folium*. *International Journal of Molecular Sciences* 19: 1-16.
- Khalid, K. and Ahmed, A. 2017. Growth and certain biochemical components of black cumin cultivated under salinity stress factor. *Journal of Materials and Environmental Science* 8: 7-13.
- Majdi, M., M. R. Abdollahi and A. Maroufi. 2015. Parthenolide accumulation and expression of genes related to parthenolide biosynthesis affected by exogenous application of methyl jasmonate and salicylic acid in *Tanacetum parthenium*. *Plant Cell. Rep.* DOI 10.1007/s00299-015-1837-2.
- Opitz, S., G. Kunert and J. Gershenzon. 2008. Increased terpenoid accumulation in Cotton (*Gossypium hirsutum*) foliage is a general wound response. *J. Chem. Ecol.* 34: 508-522.
- Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath and M. Whalen. 2010a. Natural rubber quantification in sunflower using automated solvent extractor. *Indust. Crops and Prods.* 31: 469-475.
- Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath, J. L. Brichta and J. E. van Fleet. 2010b. Agronomic and natural rubber characteristics of sunflower as a rubber-producing plant. *Indust. Crops and Prods.* 31: 481-491.
- Ramakrishna, A. and Ravishankar, G.A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior* 6: 1720-1731.
- Ravetta, D., Mclaughlin, S., and O'Leary, J. 1997. Evaluation of salt tolerance and resin production in coastal and central valley accessions of *Grindelia* species (Asteraceae). *Madroño* 44: 74-88.
- Renegasamy, P. 2006. World salinization with emphasis on Australia. *Journal of Experimental Botany* 57: 1017-1023.
- Rowe, H. C., Ro, D-K and L. H. Rieseberg. 2012. Response of Sunflower (*Helianthus annuus* L.) leaf surface defenses to exogenous methyl jasmonate. *PLoS ONE* 7(5): e37191. doi:10.1371/journal.pone.0037191.
- Seiler, G. J., M. E. Carr and M. O. Bagby. 1991. Renewables resources from wild sunflowers (*Helianthus* spp., Asteraceae). *Econ. Bot.* 45: 4-15.

- Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. New York.
- van Iersel, M. and K. Lee. 2008. Sodium chloride effects on growth, morphology, and physiology of *Chrysanthemum Xmerifolium*. HortScience 43: 1888-1891.
- Whittstock, U. and J. Gershenzon. 2002. Constitutive plant toxin and their role in defense against herbivores and pathogens. Curr. Opin. Plant Biol. 5: 300-307.